

The University of Chicago

ANATOMY AND HISTOLOGY OF  
THE EUREKA LEMON

A DISSERTATION SUBMITTED TO THE  
FACULTY OF THE DIVISION OF THE  
BIOLOGICAL SCIENCES IN CANDIDACY FOR  
THE DEGREE OF DOCTOR OF PHILOSOPHY

DEPARTMENT OF BOTANY

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# ANATOMY AND HISTOLOGY OF THE EUREKA LEMON

ERNEST S. FORD

(WITH FORTY-SEVEN FIGURES)

## Introduction

VOLKAMER (62) and RISSO and POITEAU (43) described and illustrated gross aspects of many species of *Citrus*. Their reports are of morphological significance mainly because they served as the background for the work of BONAVIA (9), who, without significant anatomical data of his own, presented an interpretation of the citrus fruit based upon comparative anatomy. He regarded the rind as one whorl of sterile carpels, while the pulp was considered a second whorl of carpels which were fertile and whose oil glands (juice sacs) protruded into the locules. Evidence for this concept of the juice sacs was found in the related genus *Oegle*, where more definite oil glands were found projecting from the pericarp into the locules. BONAVIA's concept of the two whorls of carpels is similar to SAUNDERS' (45) concept of the nature of the angiosperm gynoecium.

Phases of the macroscopic structure of the fruits of several species of *Citrus* have been described (11, 12, 25, 51, 65). ROSS (44), in describing the lemon fruit, applied the classic concept of the carpel in interpreting the different vascular bundles of the gynoecium, although he did not follow their courses. He referred to the juice sacs as carpel hairs but presented no more histological data than had GREW (25) three centuries earlier.

References to teratological flowers and fruits of *Citrus* have been discussed by PENZIG (39) and MASTERS (32). Among these are the instances of extra whorls of

carpels and the occurrence of sterile digitate carpels. The latter, in a variety of lemon, has been used by SAUNDERS (46) as evidence in favor of the theory of polymorphic carpels. In this variety only the dorsal part of each carpel elongates, while the marginal portion remains unseparated from the apex of the receptacle. Neither ovules nor locules are produced. This was interpreted as failure of the "fertile carpels" to develop while the "sterile carpels" do develop and form the anomalous structures.

PAYER (37) has presented the ontogeny of the floral parts of *C. aurantium*. PENZIG (38) has discussed histological data for the same species. He disagreed with PAYER and considered two whorls of stamens present rather than one. Also, his account of the courses of vascular bundles differs from that of VAN TIEGHEM (61) for the same species. Some histological data on *C. decumana* and *C. hystrix* were given by comparison. TSCHIRCH (58) described and figured some aspects of histology of the gynoecium and fruit of *C. vulgaris*. Both he and PENZIG (38) distinguished two kinds of carpellary emergences into the locule. The long ones, the juice sacs, were considered histologically different from, though similar in origin to, the short dome-shaped ones which had papillose cells and which TSCHIRCH thought secreted mucilaginous material into the locule. He described the formation of the oil glands. The structure of the rind has been discussed also by others

(8, 9, 14, 19, 20, 29, 38, 40) for various species.

BIERMAN (8) presented data on the cytoplasmic inclusions of the parenchymatous cells of the fruit of *C. vulgaris* and described seeds with several embryos in this and other species. OSAWA (35) dealt mainly with sporogenesis and embryogeny in *C. trifoliata* and gave some causes of the seedless condition in Washington Navel orange and in *C. nobilis*. Parthenocarpy (26), fertilization (13), and nucellar embryos (22, 23) have also been discussed. Recently data on blooming and fruit setting have been reported for several species (1, 42).

Several reports (33, 50, 51, 56) have appeared on the taxonomy and phylogeny of that section of the Rutaceae to which *Citrus* belongs. One of these (56) attempts to correlate the degree of fusion of the vascular traces, mainly those to perianth and stamens, with phyletic groups within the family and to make clear the nature of the styler canals, interpreting the gynoecial disk as a vestigial stamen whorl. In some species definite correlation between location of oil glands and ends of bundles was found.

### Material and methods

Collections were made of flowers and fruits of Eureka lemon of various ages at Lower Rio Grande Valley Experiment Station, Westlaco, Texas, by S. H. YARNELL in 1938 and by J. F. WOOD in 1941 and 1942. Certain stages were collected also at Riverside, California, by Dr. H. E. HAYWARD in 1941. The material was killed in various solutions, and serial sections were prepared by the paraffin technique. Young inflorescences, flowers, and fruits up to 15 mm. in diameter were imbedded and sectioned whole, while only suitable pieces of larger fruits were used. Considerable difficulty arose

from the presence of quantities of hesperidin raphides throughout the inner parts of fruits of certain ages. No satisfactory way of removing them was found.

### Observations

#### PEDICEL

Cymose flowers arise in axils of the leaves. Pedicels are very short and are subtended by single minute bracts. Many of the lateral flowers contain well-developed stamens but abortive pistils. These and other flowers which do not set fruit abscise at the base of the pedicel, although the petals, stamens, and pistils sometimes abscise first.

The cortex is composed of isodiametric cells and abundant air spaces. Oil glands occur frequently in the outer part of the cortex. Like those in the fruit, they begin differentiation early in the ontogeny of the organ, and new ones are formed during its maturation.

An endodermis is not well differentiated, but the transition to small cells of the pericycle usually is abrupt. The pericycle in pedicels of flowers is entirely composed of thin-walled cells, but in older stages small groups of strongly lignified cells occur adjacent to the phloem.

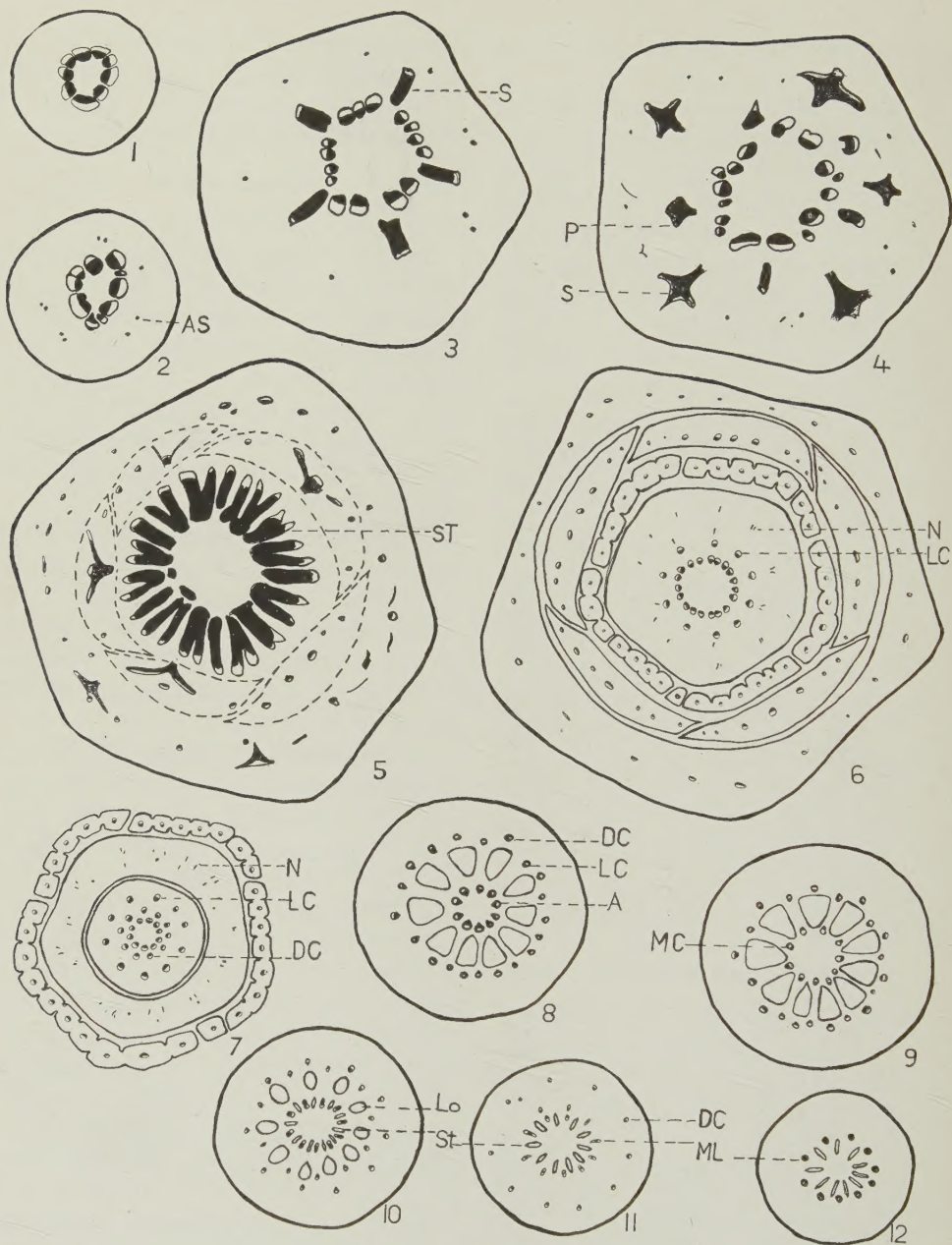
Some five to nine collateral bundles make up the vascular cylinder (fig. 1). Cambial activity is largely fascicular but finally may become interfascicular as well. Secondary thickening is not great, and most of the elements produced are not lignified, even when the fruit is half mature.

The pith usually does not exceed the width of the cortex. Its cells are isodiametric, and intercellular spaces are small. Many cells of the pith are lignified when the fruit is mature.

#### RECEPTACLE

The transition from pedicel to receptacle is gradual. The latter is consider-





FIGS. 1-12.—Figs. 1-4, cross-sections of pedicel and receptacle showing arrangement of perianth traces. *as*, accessory sepal traces; *s*, sepal trace; *p*, petal trace. Fig. 5, section showing divergence of stamen traces (*st*). Figs. 6, 7, sections at level of base of ovary showing order of divergence of nectary bundles and two lowest carpel traces. *lc*, lateral carpal (sepal) traces; *dc*, dorsal carpal traces. Fig. 8, section of ovary below divergence of marginal carpal bundles. *a*, axial or receptacular bundle. Fig. 9, section of ovary above end of residual vascular tissue showing inverted marginal carpal bundles (*mc*). Fig. 10, section above placenta showing position of stylar canals (*st*) and tops of locules (*lo*). Fig. 11, section through base of style showing convergence of marginal and lateral carpal bundles (*ml*) and position of dorsal carpal bundles (*dc*). Fig. 12, section through lower part of stigma.

ably larger in diameter than the pedicel, because the cells of the cortex and pith are larger and more numerous. There are two series of sepal traces. Below the bases of the sepals are several groups of small vascular traces. Some of these are not attached below, but others diverge from the vascular cylinder about 3 mm. below the bases of the sepals. All these bundles, accessory sepal traces (fig. 2), extend into the lateral part of the sepals. Main sepal traces diverge from the vascular cylinder at a level of bases of the sepals. In flowers with five sepals there are five main sepal traces (fig. 3), each of which has three branches. The median one of these branches is connected with the median bundles of a sepal, while the other two are curved upward and are joined with the lateral bundles of the adjacent petals.

Receptacular bundles are curved in such a way that the gaps above the main sepal traces are closed, and a whorl of petal traces diverges from the vascular cylinder at this level. These traces are equal in number to the sepal traces and alternate with them. Each petal trace has three branches (figs. 4, 5). The median one is connected with the petal bundles, while the two lateral ones extend into the marginal portions of two adjacent sepals. There is considerable anastomosis and branching of the lateral branches of the sepal and petal traces beneath the bases of sepals and petals.

A whorl of fifteen to twenty stamen traces (fig. 5) diverges from the bundles of the axis above the level of the gaps of the petals. Approximately ten of these traces are forked immediately, so that there are about twenty-five to thirty bundles which extend into an equal number of stamens. In flowers which have five sepals usually the traces of the episepalous stamen are unbranched, but

they appear at the same level as other stamen traces, not below them, as PENZIG (38) reported for orange.

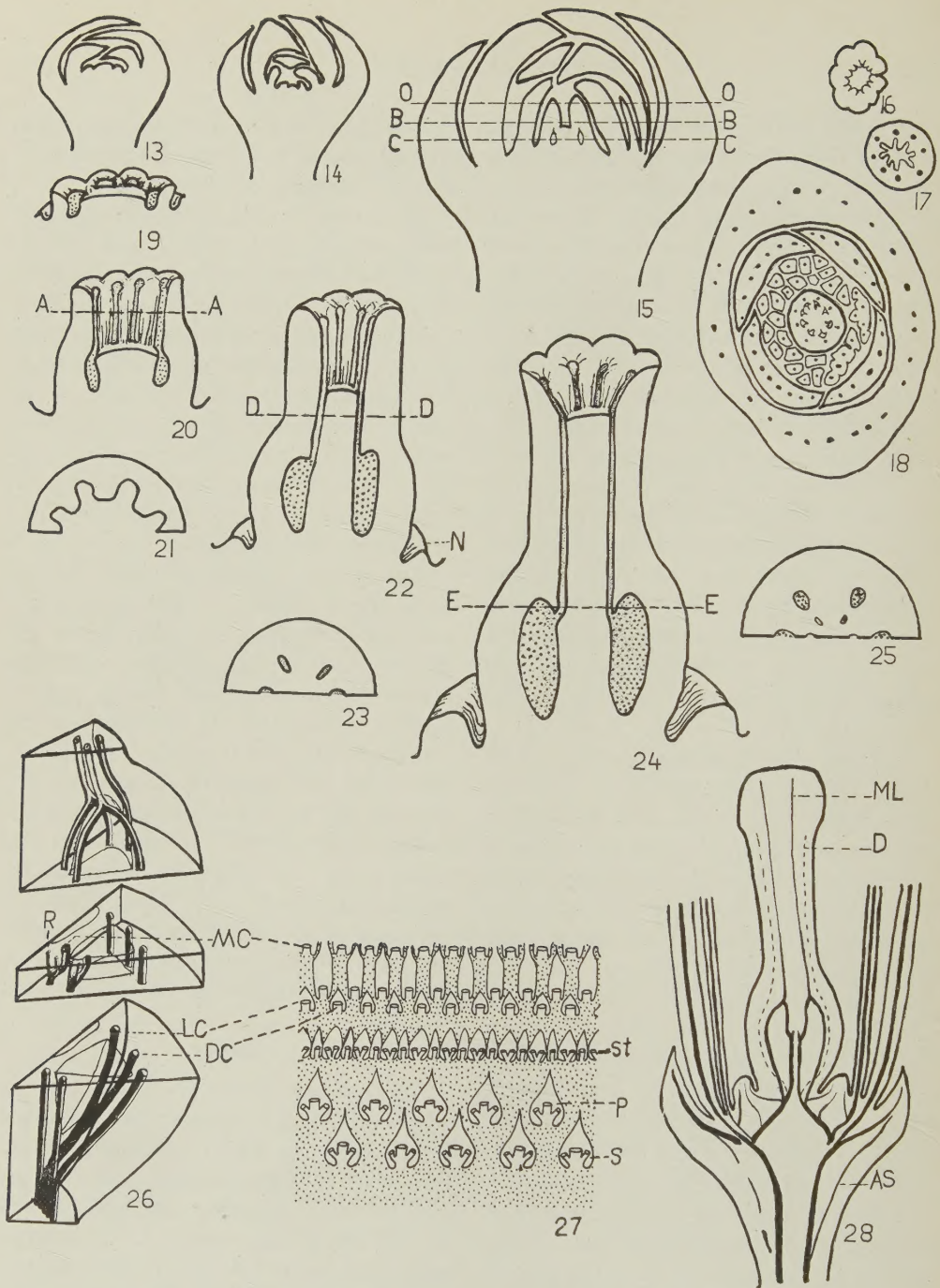
Theoretically each carpel has one dorsal bundle, two lateral or septal bundles (which are usually undiverged from those of adjacent carpels), and two similarly undiverged marginal bundles. The lateral carpel bundles diverge from the vascular cylinder just above the level of the gaps of the stamens (fig. 6). There is no specific location of these bundles in alternation with those of other floral parts below, because of the variable number of stamens and carpels.

The dorsal carpel bundles diverge from the vascular cylinder just above, and alternate with, the gaps above the lateral bundles (fig. 7). But the gaps above the dorsal carpel bundles extend several millimeters, to the level of the center of the ovary (figs. 26, 27). At this level the marginal carpel bundles diverge into positions alternate with the dorsal ones, directly inward from the septa (figs. 9, 26). The receptacular bundles terminate at the level of the divergence of the marginal carpel bundles or extend slightly above and close some of the gaps above the dorsal bundles (fig. 27).

#### CALYX

The primordia of the three to six sepals are first to appear and originate separately in a whorl, although almost immediately meristematic activity begins between the primordia, and growth of the whole zone produces the synsepalous portion of the calyx which arches over the apex of the receptacle until the developing petals force it aside (figs. 13, 14). The calyx usually is less than 1 cm. long, but its base becomes thick and broadened slightly into the saucer-shaped structure which persists when the fruit is mature.





FIGS. 13-28.—Figs. 13, 14, longisections of flower bud showing origin of stamen and carpel primordia, respectively. Fig. 15, longisection of older flower bud prior to formation of gynoeical disk. Figs. 16, 17, cross-sections of carpels at *OO* and *BB*, respectively. Fig. 18, cross-section of flower bud at *CC*. Figs. 19-25, diagrams of successive stages in development of carpels showing ontogeny of stylar canals and nectary. Fig. 21, cross-section at *AA*; fig. 23, at *DD*; fig. 25, at *EE*. Fig. 26, diagram of sector of young fruit showing arrangement of main vascular bundles of one locule. *r*, receptacular residual tissue; *mc*, *lc*, *dc*, marginal, lateral, and dorsal carpal bundles. Fig. 27, diagram representing vascular axis of flower slit lengthwise and laid out flat; position of various traces somewhat idealized. *st*, stamen traces; *p*, petal trace; *s*, sepal trace. Fig. 28, longisection of flower diagrammatically showing relation of vascular bundles. *ml*, extension of both marginal and lateral carpal bundles; *d*, dorsal carpal bundle; *as*, accessory sepal trace.



The vascular bundles of the calyx are extensions of three whorls of traces. In flowers which have five sepals the median portion of each contains branches of a main sepal trace, while bundles in the lateral portions of each are connected with branches of adjacent petal traces and with accessory sepal traces. The five to eight bundles present at the base of each sepal are branched and anastomosed sparsely along their courses through the body of the sepal. Cells of the mesophyll are compact near the apexes of the sepals but near their bases are separated by large intercellular spaces. A few oil glands occur on the abaxial sides of the sepals.

#### COROLLA

The petals originate as a whorl of crescent-shaped primordia. They alternate with the sepals and develop individually (figs. 15, 18). Their epidermal cells early become papillose, especially near the apices and edges. These papillae result in overlapping and interlocking of the petals, so that the corolla forms a closed chamber. Opening of the corolla is accompanied by shrinkage and plasmolysis of these papillose cells. In petals of a mature flower the cells of the mesophyll are isodiametric, and small intercellular spaces are abundant.

The five to eight bundles present in the base of a petal are branched repeatedly and anastomosed near the margins. These bundles are branches of two whorls of traces in the receptacle. Bundles of the median portion of each petal are connected directly with a median branch of a petal trace, while the lateral bundles of a petal are connected with lateral branches of adjacent sepal traces. While this is doubtless the fundamental pattern of vascular supply of the petals, and is in agreement with the findings of others

(38, 56, 61), great variation exists. In corollas with three or four petals several branches from two adjacent petal traces may be present in one petal. Even in corollas with five petals, which is assumed to be the ancestral state, the bundles of the lateral portion of a petal may be connected with a complex of branches of sepal and petal traces, so that the true attachment is not discernible.

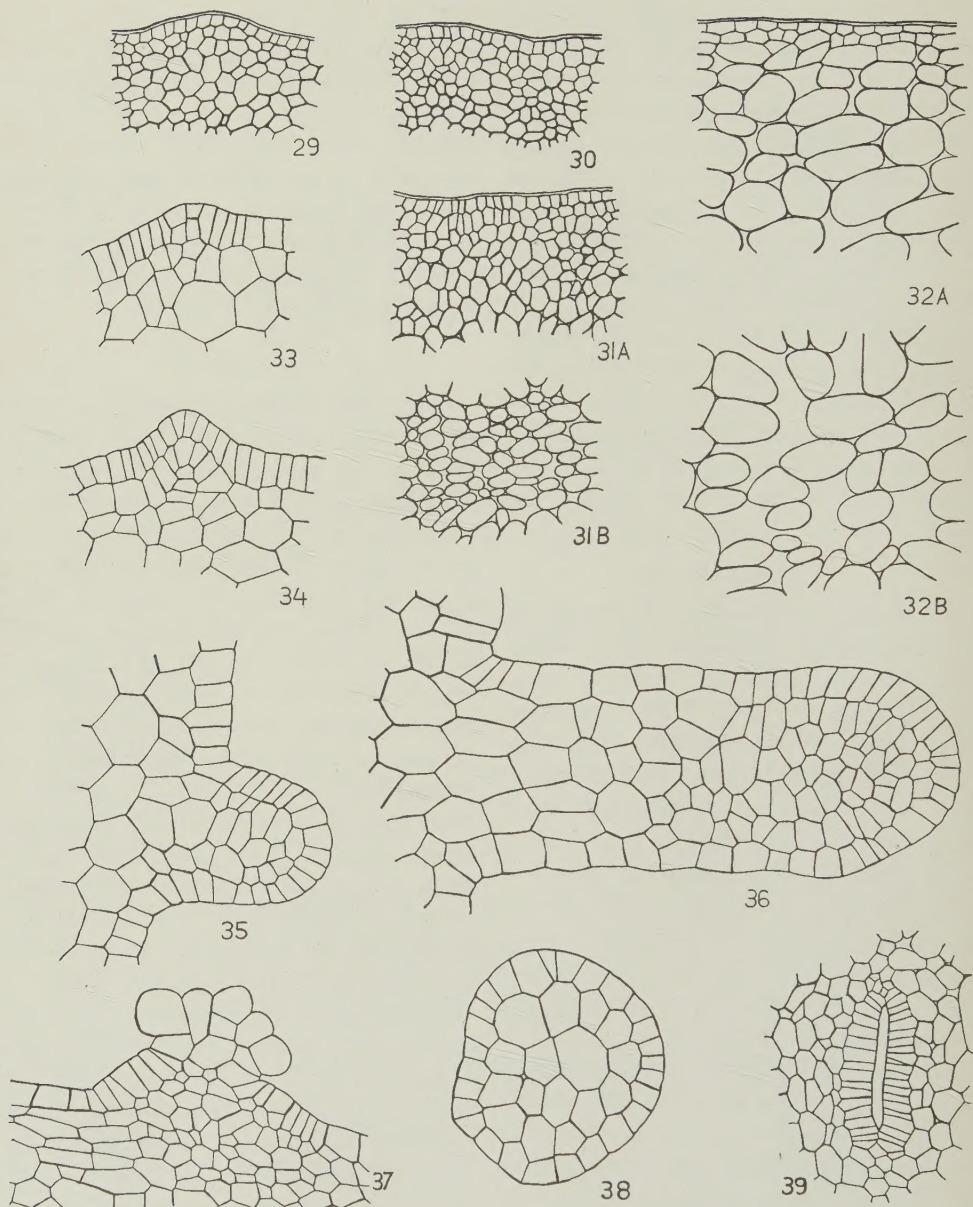
#### STAMENS

The stamens originate after the petals have grown sufficiently to arch over the apex of the axis. Stamen primordia are dome-shaped and in one whorl. Although numerous young stages were observed, there was no evidence that the primordia of episepalous stamens originate before the other ones, in the manner reported for *C. aurantium* (37, 38). While some of the twenty-five or more stamens are separate almost to their bases, others are joined laterally in groups of three to seven, and only the upper 3-4 mm. of the filaments is separated (15, 28, 48, 49). Some filaments are noticeably longer than others, but they are not correlated with definite positions.

Each stamen has a single vascular bundle which extends into the anther. In the Eureka lemon the stamen traces all diverge from the vascular axis in a zone of 12  $\mu$ , and this can hardly be conceived to be two nodes. While this observation is in accord with other data (37, 56, 61), PENZIG (38) considered the stamens of three other species of *Citrus* to arise in two whorls discernible by two whorls of vascular traces.

#### PISTIL

EARLY ONTOGENY.—The carpels originate as a whorl of about nine crescent-shaped primordia located laterally near the apex of the axis. Cell multiplication



FIGS. 29-39.—Figs. 29-32*B*, section of ovary wall and pericarp in stages from prebloom to mature fruits, with diameters of 0.65, 4, 13, and 45 mm. Figs. 31*B* and 32*B* taken from inner pericarp adjacent to dorsal carpel bundles. Figs. 33, 34, primordia of juice sacs from radial longitudinal section of ovary of unopened flowers. Fig. 35, young juice sac as seen in longisection of ovary at time petals abscise. Fig. 36, median section lengthwise of juice sac from young fruit 10 mm. in diameter. Fig. 37, median longisection of one of carpellary emergences which failed to develop into juice sac; from fruit 24 mm. in diameter. Fig. 38, cross-section of juice sac at same age as fig. 36. Fig. 39, cross-section through stylar canal of mature flower. Cf. older stage, fig. 43.



occurs between bases of the primordia simultaneously with that in the primordia and the axis in such a way that the carpels develop with their margins undiverged from the margins of adjacent carpels or the axis. In early stages, therefore, the carpels are chambers open at the top (figs. 19, 20). At first the abaxial portions of the carpels grow faster than the adaxial portions and the axis, resulting in the formation of a depression in the apex of the developing pistil (fig. 22). The terminal portions of carpels develop into the stigma, while the style is developed from the apex of the receptacle, together with the upper portions of the adjacent carpel tissue. During elongation of the carpels the tops of the chambers become constricted into stylar canals (figs. 22–25). General meristematic activity of cells of the lower portions of the carpels and adjacent axis enlarges the locules. Early in their ontogeny ovule primordia develop on both margins of the carpels, and as the locule is increased in length new primordia originate below and above those formed earlier. At the time the flower opens the pistil consists of a receptacular core undiverged from the whorl of carpels. The placentation is axile, and a stylar canal extends from each locule to the stigma.

**VASCULAR BUNDLES.**—The dorsal carpel bundles differentiate early, and they are represented by one strong bundle for each carpel. They occur in the ovary wall at positions opposite the locules and in the outer part of the style opposite the stylar canals. The dorsal carpel bundles terminate just below the stigma (fig. 28). The marginal (ventral) carpel bundles differentiate at about the same time. They diverge from the receptacular bundles at the level of the center of the ovary and may equal the number of locules (fig. 9). Marginal carpel bundles

are located in the axis of the ovary at positions opposite the septa, then are curved outward near the tops of the locules, where each anastomoses with a lateral carpel bundle (figs. 10, 11) and forms one concentric bundle which extends to the stigma (figs. 11, 12, 28). Lateral carpel bundles differentiate after the dorsal bundles. They are opposite the septa, curve inward, and are anastomosed with marginal bundles near the tops of the locules. In many instances the lateral bundles are in pairs along parts of their courses, but elsewhere the two bundles of the adjacent carpels are undiverged. The dorsal and lateral carpel bundles are much branched. These lesser vascular bundles extend outward at various angles from a complex network. Free ends of the latter bundles occur in the outer part of the ovary wall, and none of them extends into the style. At the time the flower opens the various bundles of the ovary are not well differentiated, and their histology is discussed in connection with the fruit.

**OVARY.**—When the flower opens the ovary is about 3 mm. in diameter and 5 mm. in length. The ovary wall consists of the median portions of the lower parts of the carpels and is about thirty cells thick. The outer cells of the ovary contain plastids which develop chlorophyll soon after the flower opens. Parenchyma of the middle portion of the ovary wall contains intercellular spaces but elsewhere is compact.

Oil glands are numerous in the outer part of the ovary wall. They are first detected as groups of cells whose cytoplasm is very dense and whose walls have begun to separate. Further development of an oil gland involves disintegration of a group of cells and formation of a cavity containing oil and other products. Large glands may extend halfway across the

ovary wall, as in other species of *Citrus* (8, 20, 58, 59). First initiation of glands takes place early in the development of the carpels and not after the flower is open, as reported for a Russian variety of Mandarin (20). Oil glands also occur in all other parts of the flower except the disk and the stamens, as others have noted (60). They are not associated with the adaxial surface of carpels, although BONAVIA (9) considered the juice sacs to be such.

The septa are composed of compact parenchyma and epidermis of adjacent carpels.

The lower part of the axis of the ovary consists of compact parenchyma and the receptacular bundles and placental traces. In the upper part the parenchyma is less compact, and in addition to the marginal carpel bundles stylar canals are present, the epidermal cells of which have dense protoplasts (fig. 39).

The anatropous ovules are turned in various directions. Some of their bundles diverge from marginal carpel ones, while the lower bundles usually diverge from the receptacular ones below the points where marginal carpel bundles diverge. The stylar canals are continuous with the locules above the bases of the uppermost ovules, and there are hairlike outgrowths of the epidermal cells at this point (fig. 41), each consisting of one slender cell or a filament of a few cells.

**STYLE.**—The style is composed of small unelongated parenchyma among which occur the two alternating whorls of vascular bundles, a few peripheral oil glands, and the stylar canals (figs. 11, 12). All pollen tubes observed were located in stylar canals.

**STIGMA.**—The stigma is globose and usually has an irregular central cleft. Cells at the surface are deeply staining and elongated at right angles. Near the

edge of the stigma these cells comprise only one layer, but over the apex the stigmatic surface is composed of parallel filaments of two or three cells. In some stigmas oil glands occur just underneath the surface, near the ends of vascular bundles.

#### GYNOECIAL DISK

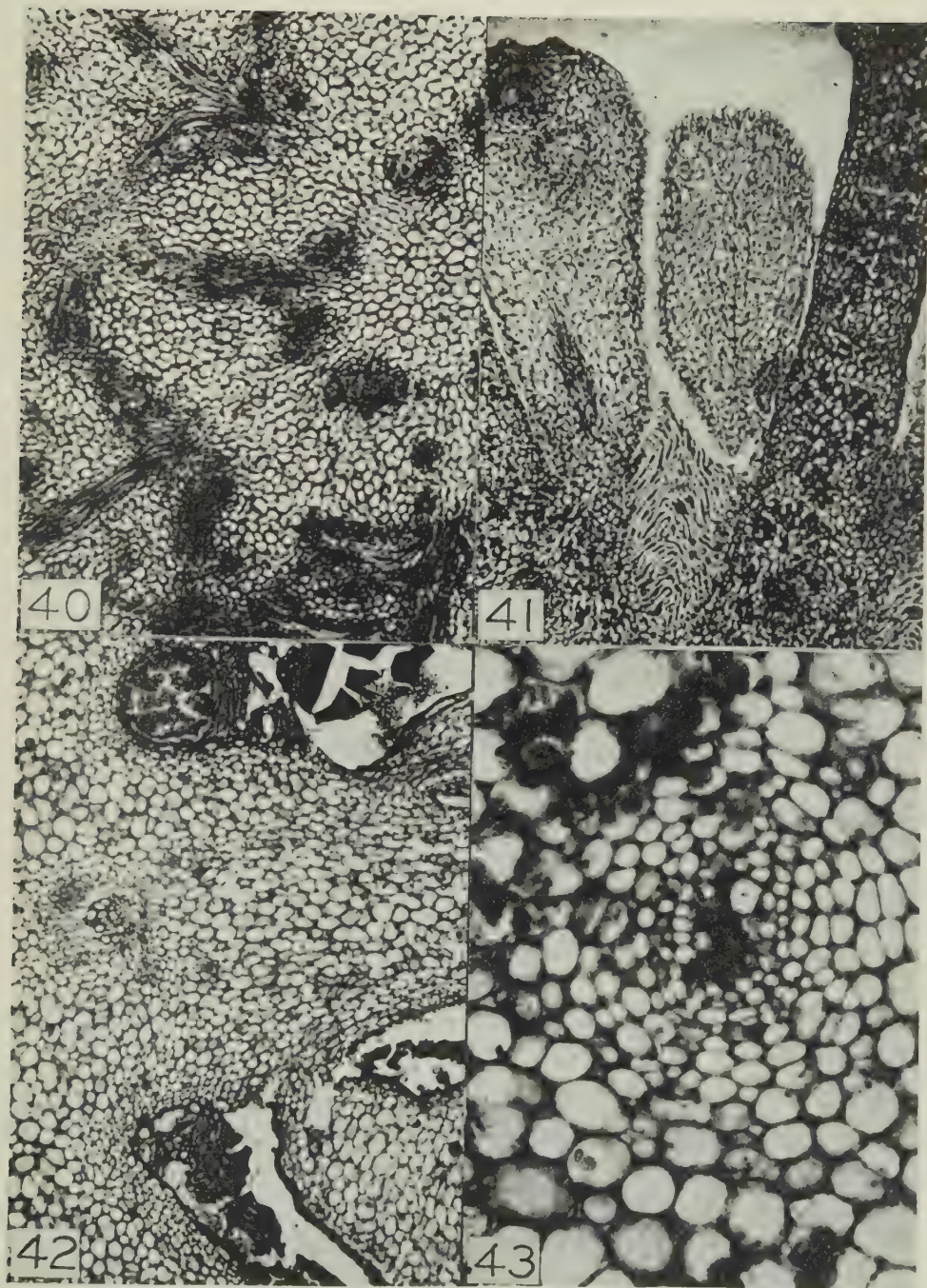
About the time the carpels begin to constrict at their tops, a ring of tissue above the bases of the stamens and below the carpels becomes meristematic and develops a massive, somewhat cup-shaped structure, the gynoecial disk, which completely encircles the base of the ovary (fig. 28). It is compact, stains densely, and its cells are exceedingly small and thin walled. Guard cells are present in its epidermis. Numerous small vascular strands are present (figs. 6, 7) and, contrary to reports (56) for other species of *Citrus*, usually are not attached directly to the vascular cylinder or to stamen traces, but to lateral carpel traces. This nectary does not develop further.

#### FRUIT

After the petals and sepals abscise, meristematic activity continues in various parts of the ovary. The top of the style disintegrates, and the fruit then develops from the ovary and base of the style (42).

**RIND.**—Cell multiplication in the ovary wall proceeds rather uniformly until the fruit is 10 mm. in diameter and the pericarp 3 mm. thick. In such young fruits the cells of the pericarp are thin walled, and intercellular spaces are minute and limited to the middle portion, where cells on the whole are twice the size of those of the outer and inner few layers. In subsequent stages the pericarp differentiates into three zones: an outer one of compact cells containing the





FIGS. 40-43.—Cross-sections. Fig. 40, of receptacle of flower bud showing one petal trace emerging from axis at position alternate with sepal traces which are branched into three strands each. Fig. 41, of ovary of open flower showing placental hairs at junction of stylar canal. Fig. 42, two undiverged inverted marginal carpel bundles. Mucilage occurs among placental hairs. Fig. 43, of stylar canal from apex of young fruit showing mucilaginous content of space. Cf. younger stage, fig. 39.

oil glands, a middle zone of spongy tissue, and an inner pericarp which is only a few cells thick and is attached to the juice sacs. Aside from the vascular bundles and epidermis, the whole pericarp is parenchymatous. The rind consists of the outer two zones of the pericarp, and although it does not separate easily from the pulp of the fruit, as does that of certain other species of *Citrus*, it is histologically distinct.

Stomata are present but are not abundant in the epidermis. Cells just underneath the epidermis are small and compact (figs. 29-31), but there is progressive increase in their size and of intercellular spaces farther inward (fig. 31*B*). These cells are isodiametric until mature, when they are slightly elongated parallel to the epidermis (fig. 32*A*). Oil glands are abundant underneath the epidermis, but they usually are separated from one another by eight or more parenchymatous cells, and many vascular bundles end near these glands. While some of the glands are formed during the early stages of development of the carpels, many new ones continue to develop, until the fruit is 15-20 mm. in diameter.

The spongy layer is notably developed (fig. 32*B*) when the fruit is 25 mm. in diameter and occupies about one-third of the width of the pericarp. Parenchymatous cells of this area are very thin walled and before the fruit is mature assume irregular shapes resembling leaf mesophyll. Intercellular spaces in mature fruits appear to equal or exceed the volumes of the cells. The demarcation between this zone and the outer one is gradual, but change to the small compact cells of the inner pericarp is abrupt.

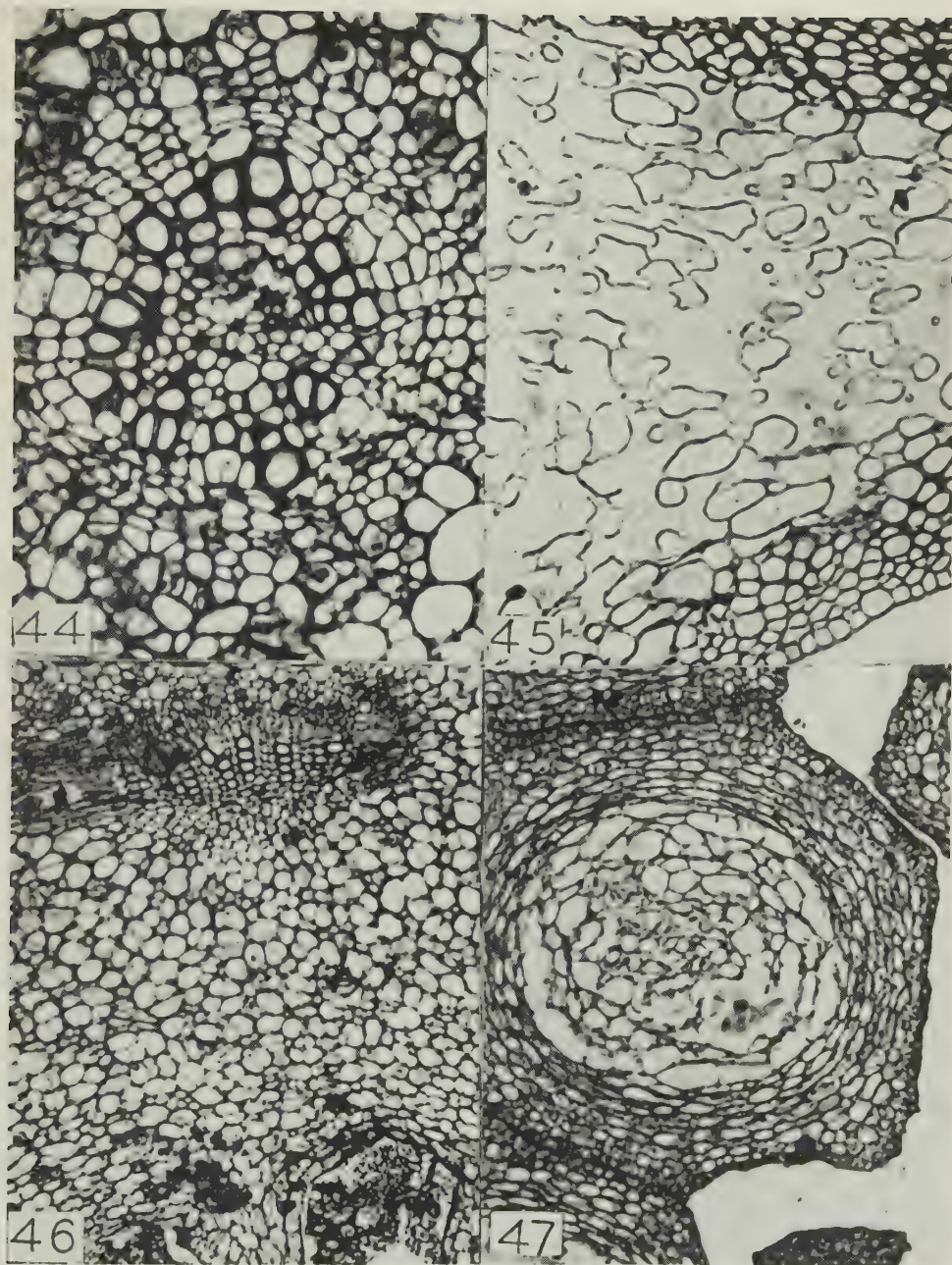
All the vascular bundles of the ovary wall occur in the rind. During development there is increase in number as well as in size and differentiation of all the

bundles. The dorsal and lateral carpel bundles change most. In young fruits these bundles are collateral and a cambium is present (fig. 46). But after the fruit is about 12 mm. in diameter the parenchymatous cells adjacent to the xylem portions of the vascular bundles become progressively meristematic toward the protoxylem of each bundle, and from their derivatives additional phloem, xylem, and cambial elements are differentiated (fig. 44).

INNER PERICARP AND JUICE SACS.—The inner pericarp is developed from the outer adaxial epidermis of the carpels, together with a narrow band of subepidermal cells. It is merged with the outer parts of the septa. The epidermal cells are similar to those of the outer pericarp, except that cutin is very thin and stomata are absent. The parenchyma adjoining the epidermis is compact and there are no oil glands. This zone contains no vascular bundles, although dorsal and lateral carpel bundles occur just outside it.

Development of the inner portion of the pericarp includes formation of the club-shaped emergences, the juice sacs, which extend into the locule. Primordia of these structures appear just before the flower opens, as reported for *C. aurantium* (41, 5). Their initiation after flowering has also been reported (18, 31). At first a primordium consists of a group of about five epidermal cells which have elongated radially and formed a small mound protruding into the locule. Primordia were observed only along the outer boundary of the locules and not on the septa. PENZIG (38) found the same condition in orange, but in *C. hystrix* he observed juice sacs attached to outer parts of the septa. In early stages of growth anticlinal divisions of epidermal cells of the primordium and division of





FIGS. 44-47.—Cross-sections. Fig. 44, of marginal carpel bundle from fruit 13 mm. in diameter. Cambium has become a cylinder and secondary xylem and phloem are present on all sides of primary xylem. Fig. 45, of inner portion of septum of mature fruit. Fig. 46, of portion of inner pericarp of 13 mm. fruit, showing position of dorsal carpel bundle and bases of juice sacs. Fig. 47, of juice sacs from fruit 24 mm. in diameter.

subepidermal cells take place. Early periclinal divisions of the epidermis were observed, but the emergence does not originate wholly from the epidermis (figs. 33, 34). During elongation of juice sacs there are repeated periclinal divisions of the epidermal cells at the tip, so that most of its cells are of epidermal origin. Young juice sacs are cylindrical and about five cells in diameter (figs. 35-38). They grow across the locules and project among the developing ovules when the fruits are 10 mm. in diameter. Each developing sac, at some point along its length, increases in diameter two or more times by cell multiplication in that area (fig. 47). This produces the familiar club shape of the emergence. There is little cell multiplication after the fruit is 20 mm. in diameter, however, and later enlargement of juice sacs results from increase in cell size. In mature fruits parenchymatous cells at the center of the juice sacs have very thin walls with minute intercellular spaces. Protoplasts have large vacuoles, and, by the use of Sudan 4, oil substances were detected in the protoplasts, as reported by DAVIS (14), as well as in the spaces formed when some cells break down. The epidermal cells which bound the mature juice sac are small, and many of them have thickened cell walls when the fruit matures.

Not all primordia develop into juice sacs, but some enlarge only slightly and their terminal cells become enlarged and globose, and cell divisions cease (fig. 37). TSCHIRCH (58) thought these papillose cells were the source of the mucilage so abundant in the locule of the young fruit of *C. vulgaris*, but Eureka lemon shows no evidence that mucilage is secreted from abortive emergences any more than from the developing juice sacs. Mucilage was not found in the locules of older fruits.

SEPTA.—Cells of the septa are markedly uniform, and cell multiplication continues until the fruit is some 20 mm. or more in diameter, when intercellular spaces become conspicuous in the median portions of the septa. This band of spongy parenchyma is merged on either side with the compact subepidermal cells and the epidermis of the carpels. In mature fruits, cells of the spongy area are stretched radially into long ellipsoidal shapes and are distinctly individual (fig. 45).

CORE.—The core develops from the axial portion of the ovary. Cell divisions increase the number to about 150 cells across the axis at the level of the placenta. In fruits that have attained 15 mm. in diameter small intercellular spaces begin to appear among the parenchymatous cells, and in subsequent development the central area of the core becomes spongy, especially above the level of the divergence of marginal bundles, where the spaces between vascular bundles is greatest. Similarly there is great development of intercellular spaces between the vascular bundles and the locules. This spongy area is continuous with that of the septa (fig. 45).

All the conducting hairs at the juncture of the stylar canals and locules become coated with mucilage and begin to decompose by the time the fruit is about 20 mm. in diameter (fig. 42). The core includes the lower parts of the stylar canals. Cells lining the canal itself usually do not break down with maturation of the fruit, but their cells become less compact. Their protoplasts become much less dense and resemble those of adjacent parenchyma (fig. 43). The canal itself contains mucilaginous material.

Vascular bundles of the lower part of the core increase in size. Fascicular cambium produces several rows of sec-



ondary tissues, and interfascicular cambium is produced between some of the bundles. Lignified tissues include a few annular elements, but for the most part they are composed of spiral tracheids 0.1–0.5 mm. in length. The thin-walled elements are elongated cells that stain densely, but no sieve plates were found. Above the ends of the axial bundles the marginal carpel bundles occur in the outer part of the core. In their early development they are endarch collateral, but during maturation they are changed by the same kind of cambial activity described previously in the dorsal and lateral bundles. The development of this condition occurs first toward the top of the locules. The ovule traces, which at blossoming time are minute, develop into conspicuous concentric bundles when the fruit is mature.

## Discussion

### FLOWER

While the flower of *Citrus* conforms with none of SHAFFNER'S (47) floral types, it is not a complex one; yet in the literature several features have led to confusion in the application of morphological criteria.

The fact that the perianth is supplied by three series of vascular bundles rather than by two from the vascular axis raises a question in regard to the accessory sepal traces. If they are considered vestiges of other flowers of the inflorescence, in agreement with the ideas of ARBER (2), then the concept of the flower as a pseudanthium is involved. Although this interpretation is conceivable, it seems unnecessary in the case of the lemon. Accessory sepal traces might be considered merely secondary bundles arising in connection with the enlarged receptacle. The fact that their provascular elements are present as early in ontogeny as those

of the receptacular bundles would not favor this idea. A third possibility is that these bundles represent lateral or marginal sepal traces. This concept is supported by the facts that they occupy normal positions for such bundles and that a few of them were found attached to bundles that diverged as main sepal traces. It may be unusual for lateral traces to diverge from the vascular cylinder below the main dorsal ones, but that condition has been described by PENZIG (38) in leaf traces of certain oranges and seems also to be true of the lateral abaxial bundles of the carpels of Eureka lemon.

It has been assumed by TILLSON and BAMFORD (56) that the stamens in *Citrus* belong to more than one whorl. PAYER (37) and PENZIG (38) thought there were two whorls in *C. aurantium*. But in Eureka lemon it has not been possible to observe either the three ages of stamen primordia or two whorls of stamen traces which the latter investigators used as evidence. The coherence of stamens in indefinite groups does not shed light on the issue, and the criterion of alternation cannot be used in this regard in lemon because of the number of carpels. In complete agreement with VAN TIEGHEM (61), observations on the anatomy of the lemon indicate there exists one whorl of stamens, although in a flower with thirty stamens they conceivably may represent two whorls of five single stamens and two whorls of five forked stamens, all "compressed" into one level. To apply WILSON'S (63) idea of primitiveness of forked stamens in the interpretation of the stamens of Eureka lemon would add to the confusion.

Opinions concerning the cup-shaped nectaries of *Citrus* have followed two widely different lines. Partly because they found its vascular bundles attached to stamen traces, TILLSON and BAMFORD

(56) considered the so-called gynoeceal disk to represent a vestigial stamen whorl. This view seems not to be substantiated by the facts that in Eureka lemon many, if not most, of the bundles present in this structure are connected with those of the main receptacle or with the lowermost carpel traces, and also that it originates after the carpels have attained considerable size. If the gynoeceal disk is a vestigial structure, there is no more evidence for considering it as derived from stamens than from another whorl of carpels. More in accord with anatomical facts is the view that the disk arises merely as a proliferation of the axis, owing to continued meristematic activity accompanying growth of the gynoeceum. This might account for the indefinite attachment of the vascular bundles supplying this structure and for its origin out of acropetal order. Views similar to this were held by BAILLON (5), PAYER (37), PENZIG (38), and others.

In the foregoing description the tacit assumptions have been that the flower represents the terminal portion of one shoot and that the carpels are foliar in nature, in the manner expressed by KOZO-POLJANSKI (30), PARKIN (36), and NEWMAN (34). Nothing has been observed in the developmental anatomy of lemon to make it desirable to apply the concept of sterile and fertile carpels of SAUNDERS (45) nor to resort to the so-called "new morphology" of THOMAS (52) or THOMPSON (53, 54, 55), who deny the existence of the carpel as such. Other contradictory interpretations regarding the angiosperm carpel are those of TROLL (57) and GREGOIRE (24), and it is not pertinent here to discuss them, for that has been done by others (4, 6, 21, 33, 64, 30, 3). GREGOIRE, however, has erred in one anatomical point, for in the fruit of Eureka lemon differentiation of the car-

pel is not different from that of a foliage leaf, either in regard to the vascular bundles or to other tissues.

How much of the gynoeceum is composed of axis tissue is of interest in species of *Citrus* because various workers, notably SAUNDERS (46) and OSAWA (35), have alluded to the prolongation of the axis in the Navel orange, and others (7, 17) have referred to this condition in other families. On evidence of ontogeny and the positions of the bundles in the style, the axis might be conceived to extend well up the style and to occupy there most of the space surrounded by the stylar canals. But there is no way to distinguish accurately how much of this tissue is carpel margins, and there is little residual vascular tissue in the Eureka lemon, and none of it extends into the style.

The epidermis of the stylar canals leads from the stigma to the placental surface of the locules. It is therefore strange, as ARBER (3) and TILLSON and BAMFORD (56) point out, that JOSHI (27) considered these structures in angiosperms generally to be vestigial vascular bundles. Because of the stylar canals, each carpel has the superficial form of a cupule whose terminal portion is constricted, as suggested by THOMAS (52) for carpels of certain simpler pistils; but this fact does not indicate homology with such structures present in the Caytoniales.

Placental areas offer no obstacle to a foliar interpretation of the carpels. The origin of younger ovules, both above and below the older ones, is explained by the general meristematic activity during development of the ovary as a whole. The continuity of the vascular bundles of the lowest ovules with the receptacular bundles is directly related to adnation of carpel margins to the receptacular axis



and does not require their interpretation as axial ovules, as BUGNON (10) did for those in *Begonia*. The hairs at the juncture of the styler canal are similar in nature to the papillose epidermal areas of the petals and the stigma.

The coherence of vascular bundles of adjacent carpels is pronounced, both as regards marginal and lateral (septal) carpel bundles, but the dorsal carpel bundles diverge from the vascular cylinder above the lateral carpel bundles. This might be expected in *Citrus*, since the traces of a foliage leaf—according to PENZIG (38)—diverge in a similar manner; that is, lateral leaf traces diverge from the vascular cylinder below the level where the main dorsal leaf traces are separate from the vascular cylinder.

The presence of concentric bundles in the style need not be confusing when it is recalled that each is an extension of two bundles; and one of these, the marginal carpel bundle, has its xylem and phloem in inverted positions because of the position of the margins of the carpels.

### FRUIT

To consider the rind of lemon a torus, as DE CANDOLLE (15) did for certain species of *Citrus*, contradicts all concepts of the angiospermous carpel except those of SAUNDERS (45), because most of the carpel bundles occur in the rind. The same may be said of BONAVIDA'S (9) concept of the rind as a whorl of sterile carpels. It is improbable that either worker conceived carpels to be polymorphic, but it seems much more likely that their ideas were based upon the ease with which the rind separates from the pulp, and that they ignored the vascular anatomy of the fruit.

When the structure of the rind is viewed in comparison with that of a foliage leaf, it becomes evident that the line

where rind can be separated from the inner pericarp coincides with the spongy mesophyll tissue present in many foliage leaves. The fact that this zone of intercellular space development in the carpels of lemon is near the upper rather than the lower epidermis can be explained on ecological bases. The separation of the sectors of the pulp from one another and from the core likewise is made possible by the copious development of intercellular spaces adjacent to the inner epidermis of the carpels. The small amount of cell disintegration observed along these lines of cleavage seemed coincidental with cell separation rather than with other causes involving secretion of specific enzymes.

It is perhaps unwise to homologize juice sacs with epidermal hairs, because this may obscure the fact that juice sacs are derived from more than the epidermis of the carpel, a fact well established in at least three species of *Citrus* by POULSEN (41), PENZIG (38), BIERMAN (8), and by the present observations. It is also improbable that the juice sacs are derived from oil glands (9). Presence of oil in cells of the juice sacs is similar to other cells of the pericarp, and the occurrence of spaces in older juice sacs is probably due to tension resulting from osmotic forces. There are no structures of Eureka lemon with which the juice sacs may be homologous.

### Summary

1. In Eureka lemon the succession of the whorls of the flower parts is acropetal. The nectary or gynoeceial disk originates after the carpels.

2. The courses of the vascular bundles and the general histological features of the floral parts are described.

3. The gynoeceium is considered a whorl of carpels around the apex of the

receptacle which extends past the level of the placenta.

4. Development of the fruit involves a period of cell multiplication throughout the ovary, followed by one of cell differentiation. Differentiation in the pericarp produces three zones: (a) an outer exocarp which is compact and contains oil glands, the flavido; (b) the mesocarp which is spongy and becomes most highly developed close to the inner pericarp, the albedo; (c) the inner pericarp which is compact and from which the juice sacs diverge.

5. Primordia of juice sacs appear just before opening of the flower and occur on the adaxial surface of each carpel. After the juice sacs grow across the locules, a portion of each enlarges greatly in diameter. Cells in the center of these areas

contain oil and are extremely delicate. Spaces containing gelatinized material in the juice sacs are considered incidental to high turgor and not to action comparable to gland formation.

6. Separation of the sectors of the pulp from one another and from the rind and core is made possible by cell separation and the formation of a spongy area in the tissue adjacent to the adaxial surfaces of the carpels.

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